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Influence of edaravone on growth arrest and DNA damage-inducible protein 34 expression following focal cerebral ischemia-reperfusion in rats

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Comments

This work explores the effect of the new agent, edaravone on GADD34 expression following focal cerebral ischemia-reperfusion in rats. The animal model is new and shows new finding that can be useful for further referencing in antioxidant research.
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ABSTRACT

Objective: To investigate the influence of edaravone on the expression of growth arrest and DNA damage-inducible protein 34 (GADD34).
Methods: A total of 108 healthy male Sprague-Dawley rats were randomly divided into sham operation group, model group and edaravone group (36 cases for each group). Transient focal cerebral ischemia was induced by middle cerebral artery occlusion for 2 h followed by reperfusion in Sprague-Dawley rats. Then, GADD34 expression was measured with immunohistochemistry at different time-points after reperfusion in the peri-infarct regions of all rats.
Results: The GADD34 expression was detected in the peri-infarct regions of rats 1 h after reperfusion, which reached its peak 24 h after reperfusion. And edaravone could significantly down-regulate the GADD34 expression.
Conclusions: Edaravon could down-regulate GADD34 expression, which suggests that edaravone may exert an important function in inhibiting endoplasmic reticulum stress reaction by scavenging free radicals in the upper stream.

KEYWORDS
Edaravone, Cerebral ischemia-reperfusion, Growth arrest and DNA damage-inducible protein 34

1. Introduction

Growth arrest and DNA damage-inducible protein 34 (GADD34) is a kind of cell cycle protein that can be up-regulated under conditions of DNA damage, cell cycle arrest and endoplasmic reticulum (ER) dysfunction, *etc*[1]. Some animal models with cerebral ischemia indicated that GADD34 expression was up-regulated at certain time windows after cerebral ischemia, but there was no consistent conclusion on the dynamic changes of GADD34 expression due to the different animal breeds and models used in the researches[2,3]. Edaravone can scavenge free radicals and is verified to have neuro-protection function in animal experiments. However, its influence on GADD34 expression has been rarely reported in recent years. In this study, edaravone was used as an interventional agent to detect the GADD34 expression changes in peri-infarct regions on ischemic parietal cortex, hoping to provide experimental basis for the prevention and treatment of cerebral infarction.

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2. Materials and methods

2.1. Materials

Edaravone was supplied by Jiangsu Simcere Company, GADD34 antibody was purchased from American Santa Cruz Company, and streptavidin-biotin complex (SABC) immunohistochemical kits and diaminobenzidine stain were brought from Wuhan Boster Biological Technology, Ltd.

2.2. Animal models and grouping

A total of 108 healthy male Sprague-Dawley (SD) rats (230-280 g) aged 10-12 weeks were fed by full-price nutritional fodders at 18-25 °C room temperature, 50%-60% relative humidity and 12 h diurnal cycle of illumination. All rats could eat and drink freely. Then they were randomly divided into sham operation group, model group and edaravone group by random number table (36 cases for each group). Suture-occluded method reported by Paschen *et al.* was adopted to prepare left focal middle cerebral artery (MCA) occlusion models[4]. The occlusion was maintained for 120 min and then unplugged to form reperfusion. After model rats revived, neurological deficit score was conducted based on the level V of Paschen's standard scoring method[4]. Rats with 1-3 scores in the initial neurological deficit score were included, whereas the dead rats, or whose with subarachnoid hemorrhage and without contralateral hemiplegia signs were considered as failed ones. Successful rat models in the same period were selected to supplement the object numbers. And then, the two groups were divided into six subgroups according to the reperfusion times such as reperfusion 1, 3, 6, 12, 24 and 72 h groups (12 cases for each group). At each perfusion corresponding time, the rats were sacrificed.

2.3. Methods

2.3.1. Edaravone group

A volume of 10 mg edaravone injection (Trade name: Edaravone; Batch number: H20031342; Specification: 10 mg/5 mL) was diluted by 5 mL normal saline to prepare 1 mg/mL solution. About 3 mg/kg edaravone injection was injected into the caudal veins immediately after reperfusion.

2.3.2. Model group

A total of 3 mL/kg normal saline was injected into the caudal veins immediately after reperfusion.

2.3.3. Sham operation group

This group was divided into six subgroups according to the above reperfusion corresponding times (six cases for each). In this group, suture occlusions were inserted 8-10 mm in depth so as to maintain the smooth of anterior and posterior MCA. The rest operations were similar to those in other groups.

2.4. Preparation of paraffin sections

At reperfusion corresponding time-point, 10% chloral hydrate was used to narcotize the rats, and then 250 mL 0.9% normal saline was infused from apex cordis. When the solution discharged from the right atrial appendage was clear, 300 mL 4% paraformaldehyde was infused. All rat brains were decollated and the part from antinion to occipital lobe was divided into five equal sections marked as A, B, C, D and E. C section was embedded by paraffin to prepare the consistent slices (4 μm in thickness).

2.5. Immunohistochemical staining

SABC was used. The potency of GADD34 polyclonal antibodies was verified to be 1:100 by the pre-experiment, and the detailed operations were as follows. The slices were routinely deparaffinaged into water, soaked in 3% H_2O_2 at room temperature for 5-10 min, and washed by double distilled water by 2 min×3 times. The antigen was repaired by microwave heating, washed by 0.1 mol/L phosphate buffer solution (PBS) by 2 min×2 times after natural refrigeration; added with normal goat serum blocking buffer, incubated at room temperature for 20 min and the serum was discarded; then added with the primary antibodies of rabbit anti-mice GADD34 polyclonal antibodies (1:100), incubated at 4 °C overnight, washed by 0.1 mol/L PBS by 2 min×3 times; added with biotinylated goat anti-rabbit IgG antibodies (carried with kits with potency 1:100), incubated at 37 °C for 20 min and washed by 0.1 mol/L PBS by 2 min×3 times; added with SABC, incubated at 37 °C for 30 min and washed by 0.1 mol/L PBS by 5 min×4 times; added with diaminobenzidine to develop color; and then dehydrated, transparentized and sealed. The above steps were performed in sequence. Five fields were randomly collected in the peri-infarct regions of ischemic cortex of each rat under microscope (×400), and the mean value was regarded as the estimated value. High-definition pathological image analysis system (HPIAS-1000) was applied to analyze the images of slices and determine the grey value of positive protein expression.

2.6. Statistical data analysis

SPSS 11.5 software was applied for all data analysis. All data were expressed as mean±SD. One-way ANOVA was used for the comparisons of multi-sample means while student's t-test was for comparisons among groups with α=0.05. P<0.05 was considered to be statistically significant.

3. Results

In sham operation group, there were positive GADD34 immunoreactive cell expressions in the left side of the parietal cortex, but ANOVA test showed no significant differences in GADD34 immunoreactive cell expressions at each time point (P>0.05). In model group, 1 h after reperfusion, GADD34 expression increased in peri-infarct regions on ischemic parietal cortex, whose grey values decreased along with the prolonged reperfusion times, indicating that the counts of positive GADD34 cells increased gradually and reached its peak at 24 h, and even 72 h after reperfusion, the positive cells were still visible. However, the GADD34 expression, marked by dynamic changes, were evidently higher in edaravone group 6, 12 and 24 h after reperfusion than in model group (P<0.05), but the differences at other time-points were not statistically significant (P>0.05), as shown in Table 1 and Figures 1 and 2.

Table 1

Comparison of mean gray values of GADD34 expression on left parietal cortex (mean±SD).

Groups	Mean gray values of GADD34					
	1 h	3 h	6 h	12 h	24 h	72 h
Sham operation group	167.77±8.55	166.39±8.08	166.54±7.74	164.62±7.40	167.06±8.11	168.61±9.35
Model group	151.72±9.92**	147.68±8.79**	125.72±7.66**	121.99±6.73**	105.99±7.02**	158.76±10.28**
Edaravone group	155.91±9.35**	152.05±11.7**	140.23±11.51**##	138.22±8.59**##	121.65±8.37**##	161.76±8.69**

**P<0.01 compared with sham operation group; ##P<0.01 compared with model group.

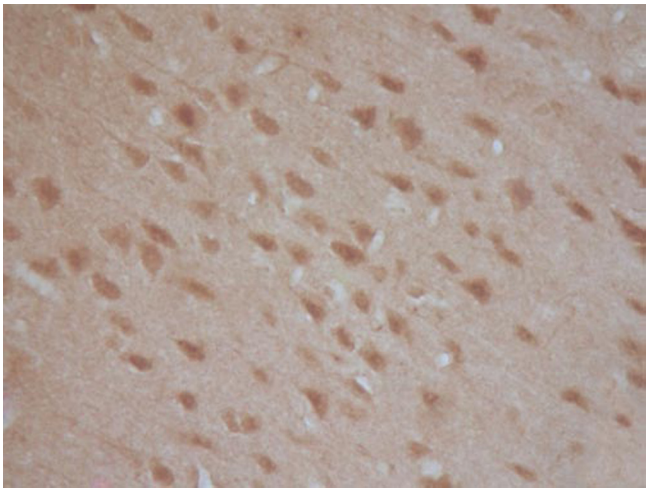
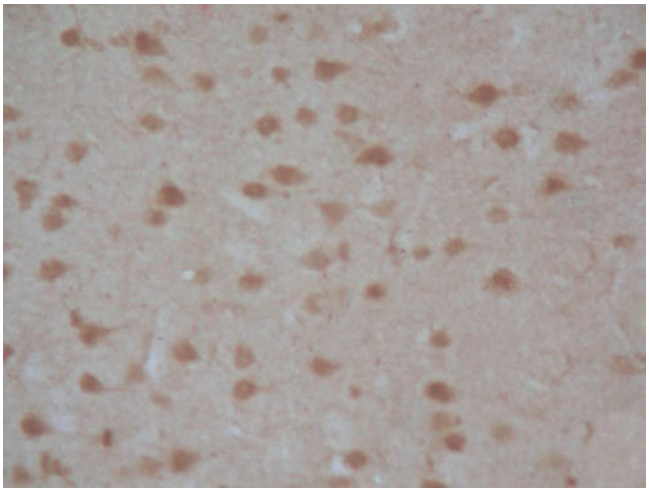


Figure 1. GADD34 positive cell expression in peri-infarct regions of left parietal cortex 24 h after reperfusion in model group.



Fgure 2. GADD34 positive cell expression in peri-infarct regions of left parietal cortex 24 h after reperfusion in edaravone group.

4. Discussion

GADD34 is a kind of cell cycle protein that can be up-regulated under conditions of DNA damage, cell cycle arrest and ER dysfunction, *etc*[4]. A recent study on *GADD34* genes in fibroblasts in rats proved that this protein could dephosphorylate eIF2α and repair endoplasmic reticulum stress (ERS) associated protein synthesis inhibition[5,6]. Therefore, it can be said that *GADD34* gene expression is limited by ERS, becoming a critical factor for the successful recovery of protein synthesis function.

According to the known functions of GADD34, it can be concluded that the increased protein compound expressions of GADD34 serve the purposes of alleviating ischemia induced protein synthesis inhibition, repairing damaged DNA and influencing programmed cell death (apoptosis)[7-10]. However, the intriguing point is that the pre-treatment of ischemia could obviously reduce the cerebral ischemia-reperfusion induced protein synthesis inhibition[11]. Garcia *et al.* reported that GADD34 was one of the proteins induced by the pre-stimulation of ischemia (5 min sub-fatal stimulation) and could be strongly translated during reperfusion after fatal ischemia, for which the pre-treatment of ischemia could remarkably protect the brains so as to response to the fatal ischemic stimulation for 30 min[11].

The existing animal experiments proved that cerebral ischemia could induce ERS and improve unfolded protein response. However, the dynamic changes were not consistent due to the different animal breeds and models used in researches. Hu *et al.* made male SD rat models with focal cerebral ischemia by electrocoagulation to observe the GADD34 expression changes at different time points after ischemia[12], and the results revealed that 4 h after operation, the ischemic brains were detected with GADD34 immune positive cells (mainly neurons). The cells were the most intensive in peri-infarct regions, and there was significant difference when compared with those in contralateral cerebral cortex. And 24 h after operation, the immune positive cell expressions reached the peak, and the double staining of GADD34 and terminal deoxynucleotidyl transferase mediated nick end labeling immunofluorescence showed deficient co-localization of neurons in the ischemic cortex. In this study, it was found that GADD34 expression, which was up-regulated at certain time after cerebral ischemia-reperfusion, could be detected 1 h after reperfusion and reached to the peak 24 h after reperfusion, which further proved that reperfusion could induce ERS and improve unfolded protein response target gene expression.

Edaravone (3-methyl-1-phenyl-2-pyrazon-5-ketone), as a new colorless transparent free radical scavenger, can effectively scavenge hydroxyl free radicals by inhibiting activities of hydroxyl free radicals, lipid peroxidation and ferric ion induced peroxidation injury. Additionally, it can also inhibit the activities of xanthine oxidase and hypoxanthine oxidase, improve the activity of superoxide dismutase, strengthen the total anti-oxidation function of cells, inhibit the peroxidation of cell membranes as well as stimulate the formation of prostaglandin, reduce the level of inflammatory factor leukotriene so as to significantly promote the cell stability, protect neurocytes, decrease cell apoptosis and increase cell survival rates. Previous study showed that edaravone had neuroprotective effect in that it could down-regulate the ERS gene expressions after cerebral ischemia-reperfusion, such as PKR-like endoplasmic reticulum kinase and C/EBP homology protein, and reduce the apoptosis of nerve cells in peri-infarct regions[13-15]. Other clinical trials also demonstrated that the mechanism of neuroprotective effect of edaravone was that it could alleviate the damage of vascular endothelial cell, inhibit the delayed neuron necrosis and apparently reduce the cerebral infarction volume injured by the obstruction-reperfusion of unilateral MCA[16-19].

This study found that edaravone could reduce GADD34 expression in SD rat models with cerebral ischemia-reperfusion, while GADD34 was verified to be effective in protecting nerves and beneficial to cell survivals. Therefore, it is controversial that whether the cerebral ischemia-reperfusion may be aggravated if GADD34 expression is inhibited by

edaravone. The cell outcome mainly depends on the strength and duration of ERS because ERS has both increased expression of apoptosis-promoting molecule C/EBP homology protein and up-regulated expressions of pro-survival molecules GADD34 and GRP78, which means that the relationship between GADD34 expression and ERS is the power comparison of survival and death. So it was predicated that, through scavenging free radicals, edaravone could evidently relieve oxidative stress in ischemic penumbra and protect ER against lipid peroxidation so as to inhibit the ERS and ERS gene expression in upper stream and play its neuroprotective effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

This is a basic laboratory study on influence of edaravone on GADD34 expression following focal cerebral ischemia-reperfusion in rats. It is a good experimental study in animal model.

Research frontiers

The work explores a new aspect on effect of the new chemical edaravone on GADD34 expression following focal cerebral ischemia-reperfusion in animal model. It is a new finding in antioxidant research.

Related reports

There is no previous report using this model for exploration on the effect of edaravone.

Innovations and breakthroughs

This is an actual innovation in antioxidant research. It is also a new report on the new agent, edaravone. The study on GADD34 expression following focal cerebral ischemia-reperfusion in rats is a new model study.

Applications

It can be further applied in the field of antioxidant research. The work can be further referenced in the field of pharmacology as well.

Peer review

This work explores the effect of the new agent, edaravone on GADD34 expression following focal cerebral ischemia-reperfusion in rats. The animal model is new and shows new finding that can be useful for further referencing in antioxidant research.

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